Tetrahedron Letters No. 1, pp. 39-46, 1963. Pergamon Press Ltd. Printed in Great Britain.

APPLICATION OF MASS SPECTROMETRY TO STRUCTURE PROBLEMS.

THE STRUCTURE OF QUEBRACHIDINE[1]

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(Received 25 September 1962)

An investigation of the alkaloids from the leaves of *Aspidosperma quebracho blanco* Schlecht. yielded, in addition to considerable quantities of aspidospermine, a new alkaloid which was named quebrachidine. It was shown to have structure (I) on the basis of arguments presented in this paper.

The alkaloid was obtained in *circa* 0.18% yield by direct crystallization (benzene) of the alkaloid fraction obtained by extraction of the alkalinized, ground leaves with benzene.[2] Quebrachidine, m.p. 276-278°, $[\alpha]_D^{26} = +54°(CHCl_3)$, $\lambda_{EtOH}^{max.}$ 242, 290 mμ; a_m 6,390, 2,920, appeared to be a dihydroindole with

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[1] Part XI. For part X see K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *J. Am. Chem. Soc.*, in press.

[2] Details of this extraction procedure have been reported for other *apocynaceous* genera: M. Gorman, N. Neuss, N. Cone and J. A. Deyrup, *J. Am. Chem. Soc.* **82**, 1142 (1960).

no aromatic substitution (four aromatic proton multiplet δ = 6.6 to 7.2 ppm.) and a second basic nitrogen (pK'_a 6.7). The infrared spectrum indicated the presence of N-H (3590 $cm.^{-1}$), O-H (3370 $cm.^{-1}$) and ester (1722, 1235 $cm.^{-1}$) moieties, while the N.M.R. spectrum showed the ester to be a carbomethoxyl (δ = 3.6 ppm.) and that an ethylidene grouping (methyl doublet centered at δ = 1.5 ppm., vinyl proton quartet centered at δ = 5.1 ppm., with J = 6.5 cps.) was present.

The empirical formula $C_{21}H_{24}N_2O_3$ could be unambiguously assigned to I from the mass spectrometric molecular weight of 352 and a consideration of the above physical measurements (N.M.R. proton count = 24). The presence of intense peaks in the mass spectrum (Fig. 1a) at m/e 130 and m/e 143 (indole nucleus with one and two carbons, respectively) supports the assumption of a dihydroindole alkaloid lacking substituents in the benzene ring and at the dihydroindole nitrogen.[3]

Acetylation of quebrachidine with acetic anhydride in pyridine afforded an amorphous diacetate (II) with the appropriate molecular weight of 436 (mass increase 84 units = 2 x 42, the mass difference of H vs. CH_3CO). One of these must be an O-acetate (1745 $cm.^{-1}$, three proton singlet δ = 1.72 ppm.) because of the large peak (A) at m/e 377 (loss of 59, i.e., $-OCOCH_3$) in the mass spectrum (Fig. 1b), and the hydroxyl group from which it is derived must be secondary since a single

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[3] For a detailed discussion of the interpretation of mass spectra of alkaloids, see K. Biemann Mass Spectrometry, McGraw-Hill Book Company, Inc., New York, N. Y., 1962, chapter 8.

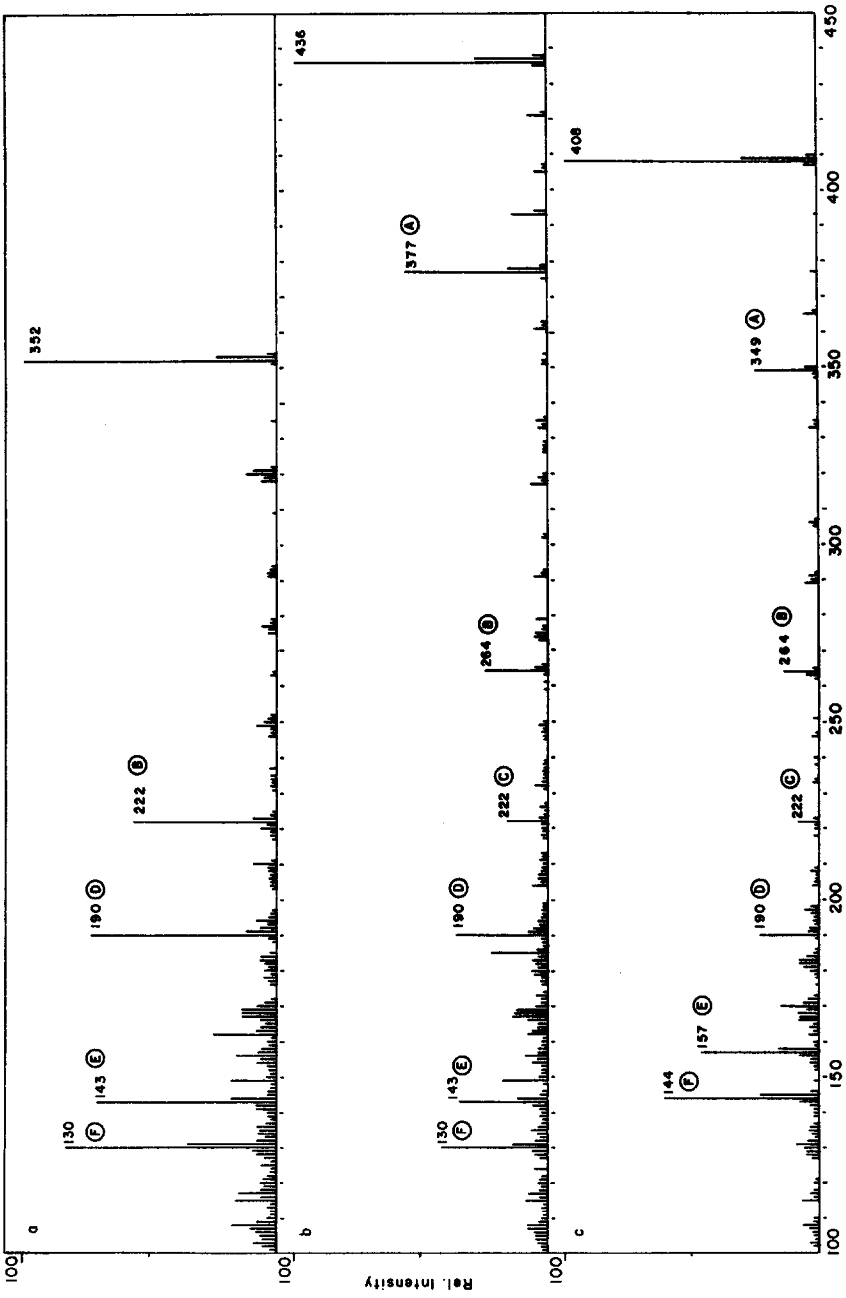

FIGURE 1. MASS SPECTRA OF (a) QUEBRACHIDINE (I), (b) O,N-DIACETYLQUEBRACHIDINE (II), AND (c) VINCAMEDINE (III).

proton in the N.M.R. spectrum of I is shifted to lower field by over two ppm. on conversion to II.[4] The second acetyl function (1658 cm.$^{-1}$, three-proton singlet δ = 2.45 ppm.) must be attached to the dihydroindole nitrogen from the accompanying shift in U.V. spectrum ($\lambda^{max.}_{EtOH}$ 250; a_m 13,950) and from the following mass spectrometric evidence: Fragment B in quebrachidine is formed by loss of 130 mass units, i.e., the dihydroindole moiety plus one carbon atom, while the equivalent (at m/e 264) in diacetylquebrachidine (II) is due to the loss of 172 mass units, i.e., 130 + 42 (H vs. CH_3CO). Fragment F is, however, still at mass 130, which shows that if this fragment retains the positive charge, the acetyl group is expelled as ketene, a typical behavior of N-acetyldihydroindoles.[1,3] The loss of the first 42 mass units, CH_2CO (to C, m/e 222), and then of 32 mass units (m.u.), CH_3OH (to D, m/e 190), also requires the presence of the acetoxy and carbomethoxy groups in fragment B.

The most revealing fact is, however, the close analogy of the mass spectra of the O,N-diacetate (II) with the one (Fig. 1c) of vincamedine (III),[5,6] which proved[3,7] that II

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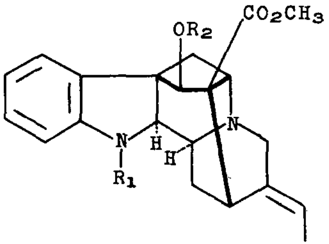
[4] A similar shift occurs in desacetylvindoline → vindoline: M. Gorman, N. Neuss and K. Biemann, J. Am. Chem. Soc. 84, 1058 (1962).

[5] J. Gosset, J. Le Men, M. M. Janot, Bull. Soc. Chem. (France) 1961, 1033.

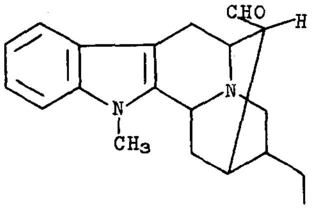
[6] M. M. Janot, J. Le Men, J. Gosset and J. Levy, Bull. Soc. Chem. (France), 1962, 1079.

[7] For a discussion of this approach, see K. Biemann, Tetrahedron Letters No. 15, 9 (1960).

has the same alicyclic carbon skeleton and that the structural difference rests in the presence of an acetyl group at N-1 in II vs. a methyl group in III. Some of the fragments (B, C and D) are of identical mass in Figs. 1b and c. These are due to the identical alicyclic moiety. Fragment A is 28 m.u. (CH_3 vs. $COCH_3$) lower in Fig. 1c while fragments E and F are 14 m.u. higher (CH_3 vs. H, because of elimination of ketene from the N-acetate II). Since structure III has been suggested for vincamedine,[5,6] O,N-diacetylquebrachidine must be II, and quebrachidine is, thus, I.



I $R_1=R_2=H$
II $R_1=R_2=CH_3CO$
III $R_1=CH_3$, $R_2=CH_3CO$



V

The correctness of these assignments was further corroborated by conversion of I into an analog (IV) of deoxyajmalal A (V),[8] which could again be correlated mass-spectrometrically. When I was formylated and then reduced with lithium aluminum hydride, a diol (VI, hydrochloride, m.p. 255-260) was obtained which on oxidation with lead tetraacetate gave an hydroxy aldehyde (VII, m.p. 212-215°). Its mass spectrum, when determined

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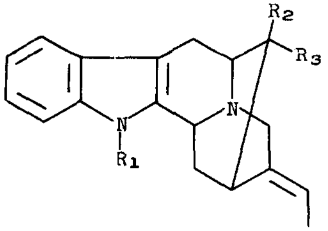
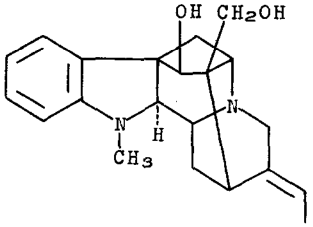
[8] M. F. Bartlett, R. Sklar, W. I. Taylor, R. L. S. Amai, and E. Wenkert, J. Am. Chem. Soc. **84**, 622 (1962).

in the conventional way (using an inlet system heated to 170°), indicated thermal loss of formaldehyde (to IV, probably the more stable exo form as shown) and clearly resembled the one of V with the exception that all peaks above m/e 250 were found two mass units lower because of the presence of a double bond in the side chain. However, when VII was introduced directly into the ion source of the mass spectrometer, the correct molecular weight (336) for the intact molecule was found. The relationship of I and III was confirmed chemically by conversion of the diol (VI) to its monoacetate of m.p. 199-201°, indicating identity with the compound of m.p. 202° derived from vincamedine.[9]



IV R_1=CH_3, R_2=H, R_3=CHO
VII R_1=CH_3, R_2=CHO, R_3=CH_2OH
VIII R_1=H, R_2=CH_2OH, R_3=CO_2CH_3



VI

Finally, when I itself was oxidized with lead tetracetate, followed by reduction of the product with potassium borohydride, a compound was obtained, the mass spectrum of which was identical with that of polyneuridine (VIII).[10]

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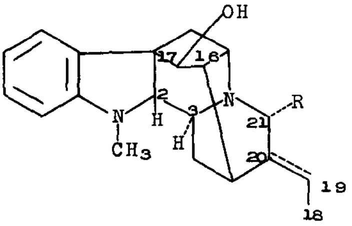
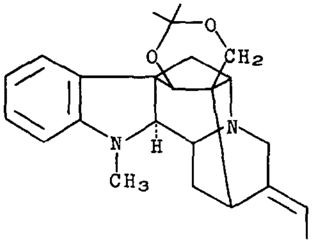
[9] M. M. Janot, J. Le Men and Christiane Fan, Compt. Rend. 247, 2375 (1958).

[10] L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, J. Am. Chem. Soc. 84, 2161 (1962).

At first, the dissimilarity of the mass spectra of quebrachidine (I), on one hand, and of tetraphyllicine (IX)[11] or ajmaline (X),[12] on the other, seemed to disagree with the above conclusions unless an assumption was made that the compounds differed in the stereochemistry at one of the carbon atoms which becomes trigonal upon oxidative opening of the five-membered ring. In fact, the mass spectrum of 2-epi-21-deoxyajmaline (2-epi-XI)[8] resembles the one of quebrachidine very closely inasmuch as it has its most intense peak at m/e 166 which corresponds to fragment B in the spectrum of I [222-58 ($-CO_2CH_3$ vs. H) + 2 (ethylidene vs. ethyl)].[13] Therefore, quebrachidine and, thus, vincamedine and vincamajine[9] (desacetyl III) have the 2-epi configuration.[14]



IX R=H, unsaturated at 19.20
X R=OH, saturated at 19.20
XI R=H, saturated at 19.20



XII

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[11] C. Djerassi, M. Gorman, S. C. Pakrashi and R. B. Woodward, J. Am. Chem. Soc. 78, 1259 (1956).

[12] R. B. Woodward, Angew. Chemie, 68, 13 (1956).

[13] A more detailed discussion of the mass spectra of compounds with the ajmaline carbon skeleton will be presented elsewhere.

[14] This has been first suggested by W. I. Taylor (private communication) on the basis of synthetic experiments.

The stereochemistry at C-17 cannot be deduced directly from the mass spectra because the spectra of 21-deoxyajmaline (XI) and its 17-epimer (17-epi-XI)[8] are almost indistinguishable. It was, however, possible to deduce this stereochemical detail in a more indirect manner. The product obtained on treatment of the diol (VI, 1 mg.) in 1 ml. of dimethoxypropane with a drop of alcoholic HCl for three hours, evaporation and generation of the free base, gave a mass spectrum (MW 378) in agreement with an isopropylidene derivative (XII) of VI. The facile formation of this derivative indicates a *cis* relationship of the hydroxyl and carbomethoxy groups in I (and therefore III) and thus the same configuration at C-17 as in ajmaline (X).

Quebrachidine (I) is the first representative of the ajmaline type to be found in *Aspidosperma quebracho blanco*, which produces, in addition, five other structural classes of indole alkaloids: namely, yohimbine, quebrachamine, aspidospermine, aspidospermatine, and eburnamemine.[15]

Acknowledgments: We are indebted to Prof. Jean Le Men for a sample of vincamedine, to Prof. R. B. Woodward for a sample of tetraphylicine, and to Drs. M. F. Bartlett and W. I. Taylor for samples of ajmaline derivatives. The work at M.I.T. was supported by a research grant from Eli Lilly and Company. Thanks are also due to Miss Ruthanne Miller for laboratory assistance and to Dr. Harold Boaz and associates for collection of physical data.

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[15] H. K. Schnoes, A. L. Burlingame, and K. Biemann, *Tetrahedron Letters*, No. 22, 993 (1962).